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BY

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From the Physiological Laboratory, Johns Hopkins University

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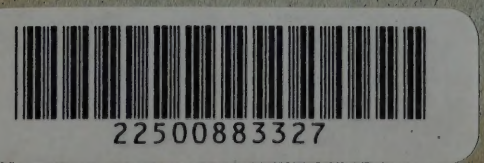

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NOTE ON THE EFFECT OF TEMPERATURE UPON THE ACTION OF THROMBIN AND ANTITHROMBIN

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The main object of this note is to call attention to the marked augmenting effect of temperatures at or above the body-temperature upon the activity of antithrombin, and also to emphasize one important condition influencing the effect of high temperatures upon thrombin.

The effect of variations in temperature upon the time of coagulation has been studied by a number of observers, both as regards the clotting of normal blood and the coagulation of artificial plasmas or fibrinogen-solutions. In the case of normal blood the conditions are quite complex since several distinct processes come into play and these processes may be affected differently by the changes of temperature, the actual time of clotting being the resultant of their interaction. We have to consider, for instance, such changes as the disintegration of the platelets, the activation of the prothrombin, the neutralisation of the antithrombin and the final reaction between the thrombin and the fibrinogen. In consequence, perhaps, of this complexity of processes, and also because of the different methods employed, the results reported by different observers for normal blood have not been entirely concordant. All observers agree that between

0°C. and 20°C. rise of temperature is accompanied by a great acceleration of the time of clotting, the temperature coefficient for a range of 10°C. being quite large, 3 to 4. Between 20° and 30°C. some observers[1] find but little difference in the time of coagulation, while others,[2] making use of more delicate methods, state that there is a marked acceleration for a rise of temperature between these points, the temperature coefficient being approximately 2.5.

Between 30° and 40°C. there is a further difference in the reported results. Brodie and Russell got no variation in time of coagulation between these temperatures, Burker and also Addis found an acceleration, the temperature coefficient being from 1.4 to 1.7. Addis whose experiments were the most extensive states that beyond 42.5°C. coagulation is delayed, the temperature coefficient becoming negative. Observations upon artificial plasmas and upon fibrinogen-solutions have not given more uniform results, although the conditions involved would seem to be less complex than in shed blood. Rettger[3] states that with fibrinogen-solutions and thrombin (prepared by the method of Schmidt) the time of coagulation remains constant for variations of temperature between 17°C. and 41°C. Landsberg,[4] on the contrary, making use of similar preparations, reports that the time of coagulation is accelerated by rise of temperature up to an optimum which lies between 37° and 40°C. Beyond 40°C. the coagulation-time is somewhat delayed. My own observations tend to confirm the results reported by Landsberg. I made use of fibrinogen-solutions prepared in the usual way from oxalated plasma and thrombin solutions obtained by a method previously reported.[5]

It may be noted in this connection that the coagulation of fibrinogen-solutions by thrombin shows sometimes irregularities

[1] Lee and White: American Journal of the Medical Sciences, 1913, cxlv, 495.

[2] Brodie and Russell: Journal of Physiology, 1871, xxi, 403. Burker: Pflüger's Archiv, 1904, cii, 65. Addis: Quarterly Journal of Experimental Physiology, 1908, i, 305.

[3] Rettger: American Journal of Physiology, 1909, xxiv, 406.

[4] Landsberg: Biochemische Zeitschrift, 1913, l, 245.

[5] Howell: American Journal of Physiology, 1913, xxxii, 264.

which it is difficult or impossible to control, especially if minimal strengths of thrombin are used. If, for example, several mixtures of the same concentrations are made and are kept in a bath at a given temperature, it may happen that one of the specimens will vary markedly in its coagulation from the time shown by the majority of the preparations. Variations of this kind are due probably to some slight undetected difference in conditions whose influence is likely to be more evident the smaller the proportion of thrombin that is used.

In general, however, it was found that the optimum temperature of coagulation lies at 35°C. or between 30° and 35°, while at 40°C. there is a tendency toward a retardation or negative coefficient, which is never very marked and may be lacking in some cases, an important condition in this respect being again the proportional amounts of thrombin and fibrinogen used in the reaction. More decisive and interesting results were obtained in experiments upon the action of thrombin upon solutions of dried calcium-free blood plasma. The mode of making and using this dried plasma has been previously described[6] and need not be repeated here. With these solutions the time of coagulation is accelerated greatly between 0°C. and 20°C., the temperature coefficient being 3 or 3+, but between 20° and 35°C. the time of coagulation is not changed perceptibly. At 40°C. there is a very marked retardation—in fact, a permanent suspension of coagulation when the amount of thrombin used is not too large. This effect may be illustrated by the following experiment in which 1 drop of the thrombin solution was used to coagulate 10 drops of the plasma.

Temperature 20°C.	Coagulation-time between 5 and 10 minutes.
Temperature 25°C.	Coagulation-time between 5 and 10 minutes.
Temperature 30°C.	Coagulation-time between 5 and 10 minutes.
Temperature 35°C.	Coagulation-time between 5 and 10 minutes.
Temperature 40°C.	No coagulation within 3 hours, during which time the temperature of the bath was retained at 40°C.

[6] Howell: American Journal of Physiology, 1911, xxix, 187 and 1912, xxxi, i; also Archives of Internal Medicine, 1914, xiii, 76.

A specimen of the last solution removed after 2 hours to a temperature of 20°C. began to clot in 30 minutes and later formed a solid clot. Landsberg has observed a similar effect at body temperature in experiments made with a thrombin solution (Schmidt's method) and a magnesium sulphate plasma. His explanation is that the thrombin is adsorbed by some protein or proteins found in the plasma. In former papers I have given evidence for the existence of an antithrombin in blood-plasma, and the thought occurred that the striking difference in reaction at 40°C. between a thrombin-fibrinogen mixture on the one hand and a thrombin-plasma mixture on the other is due probably to the antithrombin present in the plasma. Direct experiments made to test this suggestion demonstrated that it is correct. In these experiments the action of the antithrombin was determined by its effect on selected mixtures of thrombin and fibrinogen exhibiting known coagulation times. For example, oxalated human blood plasma, freshly prepared, was heated to 60°C. and filtered. The antithrombin action of the filtrate was tested upon mixtures of thrombin and fibrinogen (dried plasma) at 20° and at 40°C. according to the following schema.

20°C. Thrombin solution drops	*Heated human plasma drop*	*Time interval minutes*	*Fibrinogen drops*	*Coagulation time minutes*
4	1	15	10	4 (solid)
3	1	15	10	5 (solid)
2	1	15	10	10 (partial)

Without the addition of the drop of heated plasma similar mixtures all clotted firmly in 4 minutes.

40°C. Thrombin solution drops	*Heated humam plasma drop*	*Time interval minutes*	*Fibrinogen drops*	*Coagulation time*
4	1	15	10	10 minutes (solid)
3	1	15	10	10 minutes (partial)
2	1	15	10	No clot in 5 hours.

This result was confirmed by other similar tests. It may be concluded that in mixtures in which the thrombin is not greatly in excess of the antithrombin an increase in temperature from 20°C. to 40°C. augments the action of the antithrombin to such an

extent that coagulation is greatly delayed or entirely prevented. In the plasma of circulating blood or lymph the prothrombin and antithrombin are present in such proportions that at room temperatures the coagulation of the cell-free plasma occurs very slowly, if at all. From the results here described it is evident that in such plasmas, at the body temperature, the action of the antithrombin must be favored to such an extent that the balance will be thrown safely to its side. Under the conditions that exist in the body in which the platelets and leucocytes remain intact and in which therefore there is no sudden increase in the content of the plasma in prothrombin and thromboplastin we can understand that the permanent fluidity of the plasma will be ensured by the protective action of the antithrombin.

THE EFFECT OF HIGH TEMPERATURES UPON THROMBIN

As pointed out by Rettger[7] solutions of thrombin made by Schmidt's method may be boiled for several minutes without losing wholly their power to cause clotting in fibrinogen-solutions. On the other hand it is very well known that if serum containing thrombin is heated to 60° the thrombic power is destroyed and when oxalated plasma is brought to the same temperature the prothrombin is destroyed or so changed that it can no longer be activated to thrombin. In the thrombin that I have prepared by acetone precipitation from saline solutions of washed fibrin I have had occasion to test at various times the degree of its thermolability, with the result that in some cases it has been destroyed apparently at relatively low temporatures, while in other cases even boiling has not removed entirely its characteristic action. Examination of these results has shown that the effect of high temperatures on the thrombin is greatly influenced by the character and amount of the salts present in solution.

Sodium chloride has a marked influence in this respect as is shown by the following experiments. A specimen of dry thrombin was dissolved in distilled water to make a 0.1 per cent solution. This solution was divided into three parts: A, con-

[7] Rettger: American Journal of Physiology, 1909, xxiv, 406.

taining no salt; B, containing sodium chloride to a strength of 0.5 to 1 per cent, and C, containing sodium chloride to a strength of 1 to 1.5 per cent. The three specimens were placed in a water-bath and heated to 60°C. for two minutes. Their action was then tested upon a freshly-prepared solution of fibrinogen in comparison with the same solutions unheated.

Fibrinogen Solution, 10 drops + Thrombin, Solution A, unheated, 2 drops gave clot in 4 minutes.

Fibrinogen Solution, 10 drops + Thrombin, Solution A, heated, 2 drops gave clot in 20 minutes.

Fibrinogen Solution, 10 drops + Thrombin, Solution B, unheated, 2 drops gave clot in 4 minutes.

Fibrinogen Solution, 10 drops + Thrombin, Solution B, heated, 2 drops gave clot in 60 minutes.

Fibrinogen Solution, 10 drops + Thrombin, Solution C, unheated, 2 drops gave clot in 4 minutes.

Fibrinogen Solution, 10 drops + Thrombin, Solution C, heated, 2 drops gave clot in 95 minutes.

The same solutions were than heated to boiling over the flame for a minute and again tested upon the solution of fibrinogen.

Fibrinogen Solution, 10 drops + Thrombin, Solution A, unheated 4 drops gave clot in 3 minutes.

Fibrinogen Solution, 10 drops + Thrombin, Solution A, boiled, 4 drops gave clot in 25 to 30 minutes.

Fibrinogen Solution, 10 drops + Thrombin, Solution B, boiled, 4 drops gave no clot in 24 hours.

Fibrinogen Solution, 10 drops + Thrombin, Solution C, boiled, 4 drops gave no clot in 24 hours.

It appears from these and similar experiments that an aqueous solution of pure thrombin free from salts is weakened but not destroyed by boiling, but that the presence of sodium chloride to a concentration of 1 per cent, more or less, renders the thrombin much more sensitive to high temperatures and effects its complete destruction at a temperature of 100°C.

No visible coagulation of the solutions was produced by the heating and the way in which the salt exerts its influence upon

the thrombin remains undetermined. It may be added that boiling aqueous solutions of thrombin, free from salts, for 5 minutes, is not sufficient to destroy their action completely. They still cause clotting of solutions of fibrinogen although the time is delayed. If the solutions are kept in a bath of boiling water for 30 minutes some remnant of activity is still maintained, for when added to solutions of fibrinogen they cause, after some time, a delicate membraneous clot.

SUMMARY

1. At temperatures approximating the body-temperature the action of antithrombin is greatly augmented. It is probable that this action is of importance in ensuring the fluidity of the circulating blood in animals like the mammals in which the content of antithrombin in the blood is small.

2. The effect of high temperatures (60°–100°C.) in weakening or destroying the action of thrombin is accelerated greatly by the presence of small amounts of neutral salts (NaCl).

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